University of Alberta

Wide pulse-width, high-frequency electrical stimulation: implications for neuromuscular electrical stimulation.

by

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Abstract

Electrical stimulation using 1 ms pulse-widths, delivered at 100 Hz, produces torques larger than expected from direct motor axon activation alone. These stimulation parameters were delivered over the tibial nerve, median nerve, plantar and wrist flexors to induce contractions through direct motor axon activation and excitation of motoneurons through sensory axons (*extra contractions*). Absolute and extra contraction amplitudes, along with consistency between contractions and stability within single contractions were compared between nerve and muscle stimulation at low and moderate intensities. Extra contractions were produced in plantar and wrist flexors with nerve and muscle stimulation at each intensity. Moderate intensity muscle stimulation of the triceps surae produced the largest absolute torques and low intensity muscle stimulation evoked the largest extra contractions. Low intensity tibial nerve stimulation produced the least consistent and stable contractions. Absolute and extra contraction amplitudes were similar for all conditions in the wrist flexors.

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Chapter 1 - Review of the literature

Neuromuscular Electrical Stimulation

Neuromuscular electrical stimulation (NMES) provides numerous benefits to people with motor deficits by activating the terminal branches of the peripheral motor nerve and is used to induce therapeutic and functional motor responses in people with movement disorders (Baker et al. 2000). NMES is commonly delivered to patients recovering from central nervous system lesions such as stroke, head trauma, and spinal cord injury; orthopedic surgery including joint replacement or ligament repair; and lower motoneuron disorders including Guillian-Barré syndrome or brachial plexus trauma (Baker et al. 2000). Therapeutic NMES programs include reducing muscle atrophy through muscle strength and endurance conditioning; range of motion maintenance including contracture control; facilitation and restoration of voluntary movements; acute and chronic edema management; and spasticity reduction (Baker et al. 2000).

When the goal is to produce functional movements, NMES is referred to as functional electrical stimulation (FES) (Baker et al. 2000). FES is delivered through traditional surface electrodes and implantable stimulation electrodes placed around the nerve, near the nerve trunk, or in the muscle. Typical FES parameters consist of low frequencies and narrow pulse-widths to ensure patient comfort and minimize fatigue during continual stimulation protocols (Baker et al. 2000; Matsunaga et al. 1999). FES treatments have been implemented for a wide range of applications including foot drop stimulators, hand grasp stimulators, bipedal ambulation, and exercise equipment. Given the positive results of these treatments, FES is proving to be a viable means of muscular rehabilitation for people with movement deficits.

Electrically Induced Contractions and Motor Unit Recruitment Order

When current is applied to a nerve or a muscle, large diameter motor axons that innervate highly fatigable fast-twitch muscle fibers are preferentially recruited (Trimble and Enoka 1991). Kirchoff's law states that current will take the path of least resistance through a circuit (Baker et al. 2000). Given the inverse relationship between axon diameter and longitudinal resistance, and Kirchoff's law, larger diameter motor axons have a greater affinity for passing current versus their smaller diameter counterparts (Baker et al. 2000). As a result, larger diameter motor axons are more likely to become activated when stimulated externally. This non-physiological recruitment order is opposite to Henneman's size principle. Under physiological conditions, motoneurons are recruited with respect to diameter from smallest to largest (Henneman and Olson 1965). Physiological recruitment order is important because smaller diameter motor axons innervate more fatigue resistant muscle fibers, thereby producing more fatigue resistant voluntary contractions (McPhedran et al. 1965). NMES/FES is effective in evoking contractions in paralyzed muscle; however, recruitment reversal may contribute to rapid fatigue and compromised functionality compared to voluntary contractions.

Traditional NMES stimulation parameters are not conducive to physiological motor unit recruitment. Veale et al. (1973) demonstrated that sensory fibers have lower excitation thresholds than motor fibers when pulse widths exceed 0.5 ms. The threshold difference is based on a greater excitation time constant in sensory axons compared to motor axons (Panizza et al. 1998). Sensory axons therefore require longer pulse widths to reach threshold. Activation of sensory afferents is necessary for preferential recruitment of smaller diameter motor axons. Most FES systems use stimulation pulse

widths between 200 and 400μs (Baker et al. 2000), and are more likely to drive contractions primarily through motor axons.

Traditionally, it has been suggested that NMES preferentially recruits the largest axons first, and hence the most rapidly fatiguing muscle fibers (Salmons et al. 2004; Trimble and Enoka 1991). However, factors beyond axon diameter play a role in how motor units are recruited during transcutaneous current application. Using 1ms pulse-widths, Feiereisen et al. (1997) demonstrated a recruitment reversal in 28% of the motor unit pairs recruited in tibialis anterior. Similarly, median nerve stimulation showed no evidence of recruitment order reversal in six out of seven subjects with chronic spinal cord injury (Thomas et al. 2002). Recruitment reversal in these studies suggest factors such as location of terminal axon branches in the current field can also affect motor unit recruitment order (Knaflitz et al. 1990; Gregory and Bickel 2005). The apparent discrepancy regarding recruitment order during electrical stimulation in the literature could be explained by variations in muscles tested and experimental procedures (Feiereisen et al. 1997). Additionally, assumptions about axon biophysics are based on percutaneous, not transcutaneous, axon stimulation (Thomas et al. 2002). It is important to recognize that motor unit recruitment order during transcutaneous electrical stimulation is not solely predicated on biophysical properties.

Increased fatigue of electrically induced contractions, compared to voluntary contractions, is commonly attributed to the preferential activation of fast-twitch muscle fibers, and hence a reversed recruitment of contributing motor units (Gregory and Bickel 2005). A major difference between voluntary and electrically evoked contractions is that during a voluntary contraction, additional motor units are recruited to replace initial units

that have become fatigued; this is known as motor unit substitution (Person 1974; Westgaard and De Luca 1999). Due to the synchronous recruitment of motor units during electrical stimulation, this substitution is unable to occur, and force production declines as a result (Gregory and Bickel 2005). Thus, strategies beyond recruitment order employed by the nervous system to combat fatigue are absent during electrically evoked contractions, and fatigue is increased as a result.

Central Contributions to Electrically Evoked Contractions

Collins et al. (2001) demonstrated that electrical stimulation over the triceps surae with high frequencies (100 Hz) and wide pulse widths (1 ms) is capable of producing contraction forces beyond those expected from conventional FES. The efficacy of these stimulation parameters was confirmed in a later study (Collins et al. 2002). Pulse-widths of 1 ms generated significantly more force than .05 ms during a 7 s stimulation train at 100 Hz, and frequencies of 100 Hz generated significantly larger forces than 25 Hz. Collins et al. (2001) showed the contractions result from central and peripheral mechanisms. The peripheral component is caused by the electrical activation of motor axons directly underneath the stimulating electrodes, as with traditional FES. The central component refers to synaptically recruited motoneurons in the spinal cord from electrical activation of sensory axons. The corresponding motor volley travels down the efferent nerve fiber and causes the muscle fibers to contract. Transmission through spinal pathways is essential to the development of the large contractions, as confirmed during an experiment were lignocaine was administered to block the nerve proximal to the stimulation site (Collins et al. 2001). Prior to the nerve block, a 7s stimulation train delivered at 100 Hz (1 ms pulse widths) produced large, non-linear, force profiles. After

the nerve block, given the same stimulation protocol, the large force profiles were abolished. In the case of the nerve block, only the peripheral component could contribute to the contraction. Furthermore, large contractions could be evoked at stimulation intensities below motor axon activation threshold (Collins et al. 2002). This provides further evidence the evoked responses were of central origin as the motor axons were not directly activated.

In addition to large contraction amplitudes, Collins et al. (2001, 2002) observed sustained electromyographic (EMG) activity beyond the stimulation period. The prolonged activity was later equated with Hoffman (H-) reflexes during the stimulation period (Nozaki et al. 2003). The H-reflex is evoked by stimulating large diameter, low threshold, Ia afferents projecting from the muscle to the spinal cord (Misiaszek 2003). Excited afferents synapse monosynaptically onto α-motoneurons in the spinal cord, and the subsequent efferent volley produces a response in the muscle (Misiaszek 2003). The H-reflex is described as monosynaptic, however; oligosynaptic Ia pathways, Ib afferents from Golgi tendon organs, group II muscle spindle afferents, and large-diameter cutaneous afferents also contribute to the response (Misiaszek 2003). Spinal pathways traversed by H-reflexes are similar to mechanically evoked stretch reflexes, but due to direct activation of sensory afferents; γ-driven changes in muscle spindle sensitivity do not affect afferent input (Misiaszek 2003).

Nozaki et al. (2003) used high-frequency (50 ms), wide pulse-width (1ms), electrical stimulation over the tibial nerve, to generate self-sustained EMG activity in the soleus muscle. Stimulation of the nerve in the popliteal fossa produced clear H-reflexes during the stimulation trains at intensities below motor threshold. Given the presence of

H-reflexes during the stimulation, and the low stimulation intensity at which they were evoked, the contractions observed were said to be the product of spinal pathway excitation.

Mechanisms of Central Contributions To Electrically Evoked Contractions

Various mechanisms could be responsible for the large contraction forces and sustained muscle activity described above. Collins et al. (2001, 2002) and Nozaki et al. (2003) have proposed that plateau potentials in spinal neurons play a major role. Motoneurons were traditionally thought to be passive integrators of synaptic inputs where a linear input-output relationship determined motor output (Granit et al 1966). Current evidence shows intrinsic motoneuron membrane properties, such as low-voltage activated Ca^{2+} channels, are responsible for persistent inward currents (PICs) which produce depolarizations and discharge rates outlasting the initial input (Hultborn 2002; Bennett et al. 1998). The prolonged depolarizations caused by PICs are known as plateau potentials, and neuromodulators from the brain stem, such as serotonin (5-HT) and norepinephrine (NE), can facilitate them (Bennett et al. 2001). Both Collins and Nozaki observed self-sustained EMG activity caused by the high frequency electrical stimulation. This is consistent with previous studies demonstrating plateau-like firing properties in animal (Lee and Heckman 1998) and human (Kiehn and Eken 1997; Gorassini et al. 1998, 2002) motoneurons. The sustained activation is also consistent with human data demonstrating that under conditions of constant descending drive, high-frequency tendon vibration can recruit additional motor units (Gorassini et al. 1998, 2002). These units exhibit self-sustained firing by remaining active at levels of synaptic drive below that required for their initial recruitment (Gorassini et al. 1998, 2002). While the paired motor unit

technique used by Gorassini et al. (1998, 2002) is not a direct measure of plateau potentials in humans, it is widely accepted as strong evidence.

Post-tetanic potentiation (PTP) could also contribute to the results of Collins et al. (2001, 2002) and Nozaki et al. (2003). PTP refers to the enhancement of excitatory post-synaptic potentials (EPSPs) when evaluating motoneuronal PTP (Hirst et al. 1981). A peripheral type of PTP also exists and is described below. A proposed mechanism for PTP at the Ia synapse is a reduced probability of failure to release neurotransmitter from the presynaptic membrane, coupled with an increased likelihood of multi-quantal release (Hirst et al. 1981). Repetitive stimulation of Ia afferents has been shown to facilitate EPSPs of motoneurons, and may be one factor in maintaining motoneuron excitability (Hirst et al. 1981). Hence, PTP may account for large forces and residual EMG activity after high-frequency conditioning stimuli.

Regardless of the specific central mechanisms underlying the results of Collins et al. (2001, 2002) and Nozaki et al. (2003), the important feature for NMES is both mechanisms reside within the spinal cord. This is important because it demonstrates high-frequency, wide pulse-width, electrical stimulation utilizes spinal pathways to evoke muscular contractions in humans.

Possible Peripheral Contributions to Electrically Evoked Contractions

Peripherally, two mechanisms could contribute to increased torque after a bout high-frequency stimulation. In addition to working at the Ia synapse, PTP also exists at the muscle level and is defined by enhanced torque profiles after tetanic stimulation (O'leary et al. 1997). PTP of the muscle fiber is attributed to greater myosin light chain phosphorylation caused by increased Ca^{2+} release from the sarcoplasmic reticulum

(Abbate et al. 2000; Duchateau and Hainaut 1986; Grange et al 1993). A mechanistically similar process referred to as the catch-like property of muscle has been shown to increase contraction forces after repetitive stimulation as well. The catch-like property is induced with two initial stimulus pulses in rapid succession (~ 5ms), followed by a lower frequency constant stimulus train (Binder-Macleod et al. 1998; Ding et al. 2003). The rapid initial pulses are said to augment contraction force by taking up slack in the series elastic component of the muscle (increase muscle stiffness), and by increasing Ca^{2+} release by the sarcoplasmic reticulum and thus facilitating excitation-contraction coupling mediated by actin-myosin cross bridge formation (Parmiggiani and Stein 1981; Duchateau and Hainaut 1986).

Summary

Utilizing spinal pathways to electrically evoke muscle contractions is preferable to contractions evoked by activation of peripheral pathways alone. As mentioned previously, transcutaneous electrical stimulation can recruit motor units in descending order of axonal diameter from largest to smallest. The result is an increased recruitment of more fatigable muscle fibers, and more rapidly fatiguing contractions. When motor units are recruited via spinal pathways, a physiological pattern of recruitment is followed (Henneman and Olson 1965). These contractions will fatigue less as the smallest diameter motor axons innervate the most fatigue resistant fibers (McPhedran et al. 1965). This line of reasoning provides support for using stimulation parameters outlined by Collins et al. (2001, 2002) in applications were transcutaneous current application is used to evoke functional muscle contractions. In addition to the nerve block results by Collins et al (2001, 2002), H-reflexes recorded by Nozaki et al. (2003) confirm synaptic

motoneuronal recruitment as the most fatigue resistant motor units dominate the soleus H-reflex (Buchthal and Schmalbruch 1970). Furthermore, plateau potential involvement supports the synaptic recruitment of motoneurons, as self-sustained firing is manifested most strongly in small diameter motoneurons (Lee and Heckman 1998). Through activation of spinal circuitry, high-frequency, wide pulse-width, electrical stimulation demonstrates how electrically evoked contractions can follow a physiological recruitment order. Implementation of such stimulation parameters may improve the rehabilitation capabilities of contemporary therapeutic and functional NMES systems.

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Chapter 2 - Paper

Introduction

Neuromuscular electrical stimulation (NMES) is commonly used to restore or maintain motor function for people with movement disorders. NMES is typically delivered with large electrodes placed on the skin above the muscle belly, and contractions are evoked by activating the terminal branches of motor axons (Kukulka 1994; Baker 2000). NMES normally involves frequencies between 20 and 50 Hz and pulse-widths between 200 and 400 μs (Bowman and Baker 1985). Pulse widths ≤ 200 μs recruit motor axons preferentially to sensory axons (Veale 1973; Panizza et al 1989, 1992). Thus, traditional NMES is more conducive to direct activation of motor axons and less likely to evoke contractions through reflex pathways by stimulating sensory afferents. The electrical activation of motor axons recruits motor units in a disorderly manner, with a tendency towards a reversal of voluntary motor unit recruitment order where large diameter motor axons innervating highly fatigable fast-twitch muscle fibers are recruited first (Salmons 2004; Trimble and Enoka 1991). The problem for NMES is contractions evoked in this manner fatigue rapidly and recruitment of slow twitch muscle fibers occurs only at high stimulation intensities.

A potential solution to the fatigue associated with NMES is to evoke muscle contractions by activating motoneurons through reflex pathways. Similar to voluntary recruitment, motor units recruited via spinal pathways proceed in an order from smallest to largest diameter (Henneman and Olson 1965). Contractions should be more fatigue resistant than those evoked by direct motor axon activation, as the smallest diameter motor axons innervate the most fatigue resistant muscle fibers (McPhedran et al 1965;

Henneman and Olson 1965). Reflexive muscle activation with electrical stimulation can be achieved by optimizing the stimulation parameters to recruit sensory afferents using pulse widths between 0.5 and 1 ms (Veale 1973; Panizza et al 1989, 1992). A unique feature of contractions evoked reflexively through sensory afferents is that torque climbs more than expected by motor axon stimulation as the frequency is increased, and remains elevated when the stimulation frequency is reduced. Contractions evoked in this manner, using wide pulse widths (1 ms) and high frequencies (100 Hz), can produce up to 5 times more torque than expected from direct activation of motor axons alone (Collins et al. 2001, 2002). These "extra" contractions likely depend on afferent input to spinal neurons as anesthetic nerve block proximal to the stimulation site abolished the extra contractions (Collins et al. 2001, 2002). Further evidence of spinal involvement is; (1) extra contractions arise at stimulation intensities below motor threshold (Collins et al. 2002), (2) residual electromyographic (EMG) activity often persists after the stimulation (Collins et al. 2001, 2002; Nickolls 2004), and (3) Hoffman (H-) reflexes are enhanced during stimulation capable of producing sustained EMG (50 Hz) and maintaining extra contractions (20 Hz) (Klakowicz et al. 2004; Nozaki et al. 2003). The presence of H-reflexes during high frequency stimulation supports the idea of reduced muscle fatigue during NMES as fatigue resistant motor units dominate the soleus H-reflex (Buchthal and Schmalbruch 1970). An electrically induced physiological recruitment order via spinal reflex pathways may be beneficial for NMES applications where prolonged contractions are required for functional tasks (Collins et al. 2001). Additionally, the recruitment of slow twitch muscle fibers may be useful in recruiting portions of paralyzed muscle not recruited by direct motor axon excitation to counter muscle atrophy (Collins et al. 2001).

The purpose of this study was to determine the optimal technique to evoke contractions enhanced through spinal pathways (*extra contractions*) for NMES. To be useful for NMES the contractions must be consistent from one contraction to the next, and stable within each contraction. Identifying extra contractions in muscles of the arm could extend the application to NMES devices for the upper limb. Studies using high frequencies and wide pulse-widths have delivered stimulation directly over the muscle (Collins et al. 2001, 2002; Nickolls et al. 2004; Baldwin et al. 2004) and over the nerve trunk proximal to the muscle (Nozaki et al. 2003, Klakowicz et al. 2004, 2005; Baldwin et al. 2004). To assess the optimal stimulation site, this experiment compared contractions evoked by stimulation delivered directly over the muscle with stimulation delivered proximal to the muscle over the nerve trunk. Stimulation was delivered at two intensities to determine if a higher intensity is more effective than a lower intensity. To date, these stimulation parameters have only been tested on muscles that act about the ankle. This study will determine if extra contractions are present in wrist flexor muscles. These stimulation parameters may be useful in NMES applications where contraction fatigability is a limiting factor in the successful completion of a motor task. Furthermore, the parameters used may activate muscle fibers not normally recruited with traditional NMES and thereby improve the treatment of muscle atrophy.

Methods

Subjects

This experiment was designed to evaluate the optimal delivery of electrical stimulation, capable of evoking extra contractions that are consistent and stable, in the ankle plantar flexors and wrist flexors. Stimulation was delivered over the nerve (*nerve*

stimulation) proximal to the muscle, and over the muscle belly (*muscle stimulation*). Experiments were conducted on the lower limbs of ten subjects (8 males, 2 females; age range, 20-41 years) and the upper limbs of ten subjects (9 males, 1 female; age range, 22-41 years). Five subjects participated in both upper and lower limb experiments making the total number of subjects fifteen. Subjects were free from neurological and musculoskeletal disorders and participated after providing informed written consent. Experimental protocol was approved by the University of Alberta Human Research Ethics Board and conducted in accordance with the Declaration of Helsinki.

Protocol

Stimulation over the muscle belly is defined as *muscle stimulation*. Stimulation proximal to the muscle, over the nerve trunk, is defined as *nerve stimulation*. Both types of stimulation activate muscle fibers indirectly through the peripheral nerve. Maximal ankle plantar flexion or wrist flexion torque was measured during a 3-5 s maximal voluntary contraction (MVC) at the beginning of each experiment. Load cell measurements were sampled at 2000 Hz.

Ankle plantar flexors. Subjects were seated comfortably with the right hip and knee flexed between 90° and 110° and the right foot fixed at 90° to a stationary foot-plate with straps to hold the foot securely in place (Figure 1A). The leg was secured further with a strap just proximal to the knee. The foot-plate transferred plantar flexion torque about a 72-tooth cog wheel (22 cm diameter) attached to an S-type load cell (LCCB-500, Omega, Stamford, CT, USA) to measure the torque produced. Muscle stimulation electrodes were placed over the triceps surae. The proximal anode (15 cm × 3.5 cm) was placed midway across the gastrocnemius, approximately 12 cm distal to the crease of the

popliteal fossa. The distal cathode (8 cm × 3.5 cm) was placed over the soleus below the gastrocnemius, approximately 15 cm distal from the center of the proximal electrode. Nerve stimulation electrodes were situated on either side of the crease of the popliteal fossa over the tibial nerve with an inter-electrode distance of 1 cm. EMG recording electrodes were placed over the distal aspect of the soleus, proximal to the Achilles tendon, 1 cm apart.

Wrist flexors. The subject's right arm rested on a table with their right hand secured in a neutral position (Figure 1B). Padded blocks were fixed on either side of the wrist to reduce movement of the forearm. The hand apparatus was connected in series with a S-type load cell (SSM100, Interface, Scottsdale, AZ, USA) to measure wrist flexion torque. A taught metal chain attached to the load cell restricted movement during the contractions. A description of this device has been published previously (Carroll et al. 2005). Muscle stimulation electrodes were placed over the wrist flexors. The proximal anode (5 cm × 3.5 cm) and distal cathode (4 cm × 3.5 cm) were placed 5 cm and 12.5 cm, respectively, distal to the crease of the cubital fossa with an inter-electrode distance of 7.5 cm. Nerve stimulation electrodes were placed proximal to the medial epicondyle on the medial-distal aspect of the humerus over median nerve with an inter-electrode distance of 1 cm. EMG recording electrodes were placed as close to the flexor carpi radialis muscle as possible given the location of the stimulating electrodes, with an inter-electrode distance of 1 cm. Anatomical differences between subjects warranted small variations in stimulating and recording electrode placement on both limbs.

Stimulation

Muscle stimulation was delivered using flexible electrodes (Electrosurgical Patient Plate 1180: Split, 3M Health Care, St. Paul, MN, USA) cut to desired size. Nerve stimulation was delivered using Ag-AgCl bipolar surface electrodes (2.25 cm^2) (Vermed Medical. Inc., Bellows Falls, VT, USA).

One ms rectangular pulses were delivered using a Grass S88 stimulator connected in series with a Grass SIU5 isolator and a Grass CCUI constant current unit (Grass Instruments, AstroMed, West Warwick, RI, USA) and stimulation current was measured (mA-2000 Noncontact Milliammeter, Bell Technologies, Orlando, FL, USA). Stimulation intensity was adjusted based on peak torque responses to test trains of 5 pulses at 100 Hz. Test trains were delivered at two intensities to evoke peak torques of 2 (*low intensity*) and 4% (*moderate intensity*) of the pre-recorded MVC value. M-H recruitment curves were created from responses to 40 stimuli delivered to the tibial and median nerves at intensities ranging from sub-threshold for any response, up to those 2 times the threshold for a maximal motor response (M_{max}).

The stimulation protocol is illustrated in Figure 1C and consisted of two stimulation patterns; (1) 5 test trains delivered 5 seconds apart, and (2) five burst stimulations 10 seconds apart consisting of 20 Hz for 2 seconds, 100 Hz for 2 seconds, and 20 Hz for 3 seconds as adopted from Collins et al. (2001, 2002) and Klakowicz et al. (2004). The burst pattern was chosen because it allows for a comparison of torque generated during the first two seconds of stimulation, where torque is produced primarily through peripheral pathways, and the last 3 seconds of stimulation, where torque is the product of both peripheral and spinal contributions (Collins et al. 2001). This protocol

was followed for nerve and muscle stimulation at low and moderate intensities. Subjects were encouraged to remain relaxed and asked to read during the experiments to divert their attention from the stimulation.

Electromyography

EMG was recorded using self-adhesive Ag-AgCl surface electrodes (2.25cm^2 Vermed Medical. Inc., Bellows Falls, VT, USA). EMG recordings (Octopus AMT-8, Bortec Biomedical, Calgary, AB, Canada) were preamplified (x's 500), bandpass filtered (30-1000 Hz), and sampled at 2000 Hz using a 12 bit National Instruments A/D converter (Austin, TX, USA) connected to a computer running custom written Labview software (National Instruments).

Data Analysis

Four aspects of the torque profiles generated by contractions during the burst stimulations that were considered to be important for NMES, expressed as the mean within a 400 ms window around the time indicated, were quantified. (1) Absolute amplitude of the contraction produced, determined by the torque produced at 6 s into the stimulation. (2) Extra contraction amplitude, meant to represent the torque generated solely from spinal mechanisms, assessed by the difference in torque production after the 100 Hz stimulation (@ 6 s) compared to before (@ 1 s), and expressed as a % change. (3) Consistency of the contractions measured by calculating the coefficient of variation (CV) between the torques produced during 5 consecutive burst stimulations, at 6 seconds into the stimulation. (4) Stability within a contraction calculated by the CV for each contraction over the last 2 seconds of a stimulation train. Torque data were re-sampled

post-hoc at 100 Hz for analysis. Contraction profiles for each subject are expressed as % MVC.

M-waves and H-reflexes were quantified to explore the mechanisms underlying torque production during nerve and muscle stimulation. EMG was analyzed in subjects where M-waves could be clearly identified during both types of stimulation. In most cases the stimulus artifact prevented a clear measurement of the M-wave; however, it was still possible to record both wave-forms in the soleus of four subjects and wrist flexors of three subjects. Responses were measured (peak to peak) during the 20 Hz segments of the burst stimulation and normalized (% M_{max}). M_{max} was taken as the single largest M-wave response from the recruitment curve.

Statistics

Statistics were performed using Statistica (StatSoft Inc, Tulsa, OK, USA). Paired *t*-tests were used on individual and group data to detect differences in absolute torques produced by each stimulation type, and to detect significant differences in relative torques (extra contractions) within each stimulation type (torques @ 6 s vs. 1 s). Two factor repeated measures analysis of variance (ANOVA) were used on group data to determine the influence of stimulation type (nerve & muscle stimulation) and intensity (low & moderate intensity) on; (1) the size of the extra contractions, using difference values calculated by subtracting the torque produced at 1s from 6s into the stimulation; (2) the consistency between contractions and the stability within a contraction, using the CV values (described above); and (3) to compare the relative size of extra contractions between the plantar flexors and wrist flexors, using the difference values, generated by nerve and muscle stimulation at low and moderate intensities. Significant main effects

and interactions were tested post-hoc using Tukey's honestly significant difference test (HSD). Descriptive statistics are expressed as means (SD), and an α level of 0.05 was used to evaluate statistical significance. Figures depict data as means ± standard error (SE).

Results

Electrical stimulation was delivered over the plantar and wrist flexors with parameters known to induce contractions through direct motor axon stimulation and excitation of spinal neurons through stimulation of primary afferents. Absolute and extra contraction amplitudes, along with the consistency between successive contractions, and stability within a single contraction were compared between nerve and muscle stimulation at two intensities. Extra contractions were produced in the plantar and wrist flexors with nerve and muscle stimulation at low and moderate intensities. Moderate intensity muscle stimulation of triceps surae produced the largest absolute torques and low intensity muscle stimulation evoked the largest extra contractions. Low intensity tibial nerve stimulation produced the least consistent and stable contractions. Absolute and extra contraction amplitudes were similar for all conditions in the wrist flexors.

Contraction Amplitude

Plantar Flexors. Torque profiles generated during five successive contractions during nerve and muscle stimulation are shown for a single subject in Figure 2. Average torque profiles of the five test trains preceding the burst stimulations are also shown. Peak torque during the test trains was used to set stimulus intensity. Significant extra contractions were observed during all four types of stimulation; relative torque (1 s – 6 s)

increased from 1 – 3 % MVC and 3 – 17 % MVC during nerve (Fig. 2A) and muscle (Fig. 2B) stimulation, respectively, at a low stimulation intensity; and 4 – 12 % MVC and 12 – 26 % MVC during nerve (Fig. 2C) and muscle (Fig. 2D) stimulation, respectively, at a moderate stimulation intensity. Visually, distinct differences exist between the torque profiles generated with nerve and muscle stimulation. One such difference can be observed at the beginning of the stimulation, where a large initial peak in torque was observed during nerve stimulation, yet with muscle stimulation the profile remains smooth. This is best exemplified through comparing the first second of stimulation during low intensity nerve (Fig. 2A) and muscle (Fig. 2B) stimulation.

Group data for stimulation of the ankle plantar flexors are displayed in Figure 3. The torque profiles between nerve and muscle stimulation for the group differ in appearance (Fig. 3A). Similar to the single subject data in Figure 2, a sharp increase in torque appears at the beginning of tibial nerve stimulation, however, a much smoother increase in torque occurs when muscle stimulation commences. Comparisons used for statistical analysis between torques at 6 s and 1 s are displayed in Figure 3B. Muscle stimulation at low and moderate intensities produced significantly larger absolute torques, 13.6 (SD 6.2) and 23.3 (11.7) % MVC respectively, 6 s into the contraction compared to nerve stimulation at comparable low [1.5 (1.2) % MVC] and moderate [5.3 (4.5) % MVC] intensities. Torques generated prior to the 100Hz stimulation train (@ 1 s) were significantly different between low intensity nerve [1 (0.4) % MVC] and muscle [3 (1) % MVC] stimulation, and between moderate intensity nerve [3 (2) % MVC] and muscle [9 (4) % MVC] stimulation. However, torques were similar at 1 s between moderate intensity nerve stimulation [2.7 (2.4) % MVC] and low intensity muscle

stimulation [2.8 (1.2) % MVC]. Following the 100 Hz train (@ 6 s), however, significantly ($P = 0.004$) greater torques were produced with low intensity muscle stimulation [13.6 (6.2) % MVC] compared to moderate intensity nerve stimulation [5.3 (4.5) % MVC]. Nerve stimulation at the low intensity produced significant extra contractions in 3/10 individuals, and significantly increased torque from 0.6 (0.4) – 1.5 (1.2) % MVC. Muscle stimulation at the low intensity evoked significant extra contractions in all subjects and torque increased significantly from 2.8 (1.2) – 13.6 (6.2) % MVC. Moderate intensity nerve stimulation caused significant extra contractions in 8/10 individuals and significantly increased torque from 2.7 (2.4) – 5.3 (4.5) % MVC. Moderate intensity muscle stimulation produced significant extra contractions in all subjects and group torque increased significantly from 9.4 (4.2) – 22.3 (11.7) % MVC. Significant main effects were found for stimulation type ($F_{(1,9)} = 25.4$, $P = 0.0007$) and intensity ($F_{(1,9)} = 10.85$, $P = 0.009$) on group plantar flexion torque difference values between 1 and 6 seconds into the burst stimulation. At low and moderate intensities, muscle stimulation produced significantly greater torque differences ($P = 0.0004$ and $P = 0.0003$, respectively) than nerve stimulation, and hence larger extra contractions.

Wrist Flexors. Torque profiles of five successive burst stimulations, produced during nerve and muscle stimulation at both low and moderate intensities, are shown for a single subject in Figure 4. In this subject, significant extra contractions were produced by 3/4 stimulation types; relative torque increased from 1.3 – 3.2 % MVC during nerve stimulation (Fig. 4A) at a low stimulation intensity; and 5.7 – 7.9 % MVC and 4.9 – 6.9 % MVC during nerve (Fig. 5C) and muscle (Fig. 5D) stimulation at a moderate stimulation intensity, respectively. Low intensity muscle stimulation (Fig. 4B) did not

produce significant extra contractions as torque increased slightly from 2.2 – 2.4 % MVC. Qualitatively, the shape of the torque profiles for nerve and muscle stimulation appear similar to one another, as both show a smooth increase in torque at the onset of stimulation.

Group data for each stimulation condition in the wrist flexors are displayed in Figure 5. As indicated in the single subject data (Fig. 4A), the shape of the torque profiles between stimulation types are similar in the wrist flexors (Fig. 5A). Absolute torque produced 6 s into the stimulation was not statistically different between nerve and muscle stimulation at the low [2.5 (1.8) and 3.7 (3.3) % MVC, respectively] or moderate intensity [7.2 (4.3) and 10.9 (8.7) % MVC, respectively]. Low intensity nerve and muscle stimulation of the forearm flexors produced significant extra contractions in 7/10 individuals and yielded significant increases in torque from 1.7 (1.4) – 2.5 (1.8) % MVC and 2.5 (1.9) – 3.7 (3.3) % MVC, respectively. Moderate intensity nerve stimulation produced significant extra contractions in 8/10 individuals and significantly increased torque from 5.2 (3.3) – 7.2 (4.3) % MVC. Moderate intensity muscle stimulation produced significant extra contractions in 9/10 individuals and torque significantly increased from 6.6 (4.4) – 10.9 (8.7) % MVC. A significant main effect was found for stimulation intensity ($F_{(1,9)} = 6.06$, $P = 0.04$) in the wrist flexors. Moderate intensity muscle stimulation produced significantly larger torque differences than low intensity nerve stimulation ($P = 0.04$), however, there were no significant differences in the amplitude of torque differences between nerve and muscle stimulation at equal stimulation intensities.

Plantar Flexors vs. Wrist Flexors. The changes in torque produced by the stimulation, based on their difference scores, varied between muscle and nerve stimulation in the plantar flexors and wrist flexors. A comparison of Figures 3 and 5 demonstrates this trend; however, difference scores are not expressed. At the low stimulation intensity main effects for muscle group ($F_{(1,9)} = 32.5$, $P = 0.0003$) and stimulation type ($F_{(1,9)} = 26.0$, $P = 0.0006$); along with a significant interaction between muscle group and stimulation type ($F_{(1,9)} = 31.2$, $P = 0.0003$) was detected. The torque differences in the plantar flexors [10.6 (5.7) % MVC] were greater than those produced in the wrist flexors [1.2 (1.4) % MVC] with low intensity muscle stimulation ($P = 0.0003$), but were similar between the plantar flexors [0.9 (1.0) % MVC] and wrist flexors [0.8 (0.8) % MVC] with low intensity nerve stimulation. Main effects for muscle group ($F_{(1,9)} = 5.9$, $P = 0.04$) and stimulation type ($F_{(1,9)} = 29.2$, $P = 0.0004$), along with a significant interaction between muscle group and stimulation type ($F_{(1,9)} = 6.2$, $P = 0.03$), were identified for moderate intensity stimulation. Similar to low intensity stimulation, torque differences in the plantar flexors [13.8 (8.6) % MVC] were greater than those produced in the wrist flexors [4.2 (4.6) % MVC] with moderate intensity muscle stimulation ($P = 0.02$), but were similar between the plantar flexors [2.6 (3.2) % MVC] and wrist flexors [2.0 (1.7) % MVC] with moderate intensity nerve stimulation. In the plantar flexors, greater torque differences were produced with muscle stimulation, compared to nerve stimulation, at both low ($P = .0002$) and moderate ($P = 0.008$) intensities. In the wrist flexors, the differences in torque produced were similar between stimulation types at both intensities.

Consistency between contractions

Plantar Flexors. To assess the consistency between contractions, the CV between contractions produced during 5 consecutive burst stimulations, at 6 seconds into the stimulation, was calculated for nerve and muscle stimulation at both intensities (*see* Fig. 6A). Across the group, there were significant main effects for stimulation type ($F_{(1,9)} = 33.58$, $P = 0.0003$) and intensity ($F_{(1,9)} = 6.45$, $P = 0.03$) on the consistency of consecutive contractions. A significant interaction between stimulation type and intensity ($F_{(1,9)} = 6.25$, $P = 0.03$) was present also. Post-hoc analyses revealed contraction consistency (Fig. 6B) was similar between moderate intensity nerve [0.2 (0.1) CV] stimulation and muscle stimulation at low [0.1 (0.04) CV] and moderate [0.1 (0.04) CV] intensities. Low intensity nerve stimulation [0.5 (0.3) CV] produced contractions that were significantly more variable than moderate intensity nerve stimulation, and muscle stimulation at low and moderate intensities.

Wrist Flexors. Across the group, contraction consistency was similar for nerve and muscle stimulation at low [0.3 (0.2) and 0.2 (0.1) CV, respectively] and moderate [0.2 (0.1) and 0.2 (0.1) CV, respectively] intensities as no significant main effects were detected (Fig. 6C).

Stability within contractions

Ankle Plantar Flexors. To assess the stability of the torque produced within a contraction, the CV of each contraction profile over the last 2 seconds of stimulation was calculated (Fig. 7A). Stimulation of the ankle plantar flexors yielded a significant main effect for stimulation type ($F_{(1,9)} = 5.80$, $P = 0.04$). Contraction stability (Fig. 7B) was similar within each type of stimulation, irrespective of intensity. Moderate intensity

nerve stimulation [0.08 (0.06) CV] and muscle stimulation at low [0.03 (0.05) CV] and moderate [0.03 (0.03) CV] intensities were also similar; however, low intensity nerve stimulation [0.3 (0.3) CV] was significantly less stable than muscle stimulation at both intensities.

Wrist Flexors. Similar to contraction consistency, there were no significant main effects for stimulation type or intensity in the wrist flexors across the group (Fig. 7C). Contraction stability was similar for low [0.07 (0.04) CV] and moderate [0.05 (0.04) CV] nerve stimulation, as well as low [0.07 (0.04) CV] and moderate [0.05 (0.01) CV] muscle stimulation.

Electromyography

Raw EMG traces during low intensity nerve and muscle stimulation, in the lower and upper limb, for a single subject are presented in Figure 8. Traces show data over a 400 ms window, 200 ms on either side of 1 s into a single burst stimulation pattern. The traces demonstrate the presence of H-reflexes during nerve stimulation (Fig. 8A & C) and their absence during muscle stimulation (Fig. 8B & D). In the soleus, H-reflexes were larger than M-waves during tibial nerve stimulation (Fig. 8A) however; H-reflexes were absent during muscle stimulation (Fig. 8B). Across the four subjects analyzed the H-reflex [1.7 (1.7) % M_{max}] was 3.4 times larger than the M-wave [0.5 (0.3) % M_{max}] during tibial nerve stimulation. Conversely, M-waves [21 (16) % M_{max}] were an average of 15 times larger than H-reflexes [1.4 (1.0) % M_{max}] in the same four subjects during triceps surae stimulation. In the wrist flexors (Fig. 8C), H-reflexes were less frequent and M-waves more dominant during nerve stimulation, yet similar to the plantar flexors, M-waves remained during muscle stimulation while H-reflexes were reduced (Fig. 8D).

Across the three subjects analyzed the M-wave [17 (8.5) % M_{max}] was 8.5 times larger than the H-reflex [2.0 (1.7) % M_{max}] during median nerve stimulation. During wrist flexor stimulation M-waves [37 (23.6) % M_{max}] were an average of 18.5 times larger than H-reflexes [2.0 (1.4) % M_{max}] in the same three subjects.

Discussion

This experiment was designed to identify the most effective method of delivering high-frequency, wide pulse-width stimulation to evoke contractions enhanced through spinal pathways (*extra contractions*) for NMES. To be useful for NMES, the torque produced by the extra contractions must be large, consistent between successive contractions, and stable within each contraction. In the triceps surae, muscle stimulation produced contractions that were larger (up to 23 % MVC), evoked the largest extra contractions as torque was 4 times greater than expected solely from direct motor axon activation, and were more consistent and stable compared to contractions produced by tibial nerve stimulation. In the wrist flexors nerve and muscle stimulation were equally effective. Torques reached 11 % MVC and significant extra contractions produced up to 1.6 times more torque than expected from direct motor axon stimulation alone. This represents a unique finding as the presence of extra contractions has not previously been reported in the wrist flexors. The ability to evoke extra contractions in the wrist flexors may be useful in electrical devices assisting upper limb motor function. Absolute torques, central contribution amplitudes, consistency, and stability of the evoked wrist flexor torques were similar between the stimulation types. Muscle stimulation produced the larger extra contractions in the plantar flexors compared to wrist flexors; however, the

amplitude of the extra contractions was similar between the two muscle groups with nerve stimulation.

Implications

Applying the present stimulation parameters in rehabilitation devices would presumably take advantage of several important benefits, mainly associated with recruiting motor units synaptically, in their natural order. Theoretically, this should produce more fatigue resistant contractions (Collins et al. 2001). Recruitment of slow twitch muscle fibers could also be used to treat muscle atrophy as stimulation would recruit a portion of the muscle not activated with conventional stimulation parameters (Collins et al. 2001). The stimulation parameters used here may also assist in potentiating, or "warming-up," spinal motoneurons to assist in ambulatory training for people with spinal cord injury. Warm-up, characterized by increased motor unit discharge through the activation of plateau potentials at progressively decreasing membrane potential differences, is dependent upon repetitive input to motoneurons (Bennett et al. 1998b; Gorassini et al. 1999; Russo and Hounsgaard 1994; and Svirskis and Hounsgaard 1997). Such an effect would be useful in assisting motor neuron firing, and hence motor output, during repetitive/rhythmic tasks such as walking, in a nervous system devoid of descending input, as in people with SCI.

For clinical NMES applications such as functional electrical stimulation (FES), it is important the stimulation parameters used can generate extra contractions in populations most likely to benefit from such technologies. Using similar stimulation parameters to those used presently, extra contractions up to 24% of the maximal stimulated contraction force were produced by stimulating over the triceps surae in 11 of

13 subjects with varying degrees of SCI (Nickolls et al. 2004). Given the previous results in persons with SCI, the stimulation parameters used in this study appear promising for integration into devices assisting with motor function.

Nerve vs. Muscle Stimulation

A striking feature of the present results is the large extra contraction amplitudes evoked with muscle stimulation compared to nerve stimulation in the ankle plantar flexors. One difference between the two stimulation types for the plantar flexors is that M-waves dominated responses during muscle stimulation and H-reflexes dominated during nerve stimulation as shown in Figure 8. Spinal pathways have been identified as an imperative component in generating extra contractions (Collins et al 2001, 2002) and the presence of H-reflexes during high frequency stimulation lends support to this idea (Nozaki et al. 2003). Further evidence for spinal contributions during extra torque production has been demonstrated previously in our lab, where soleus H-reflexes during 20 Hz tibial nerve stimulation increased significantly after, compared prior to, 2 seconds of 100 Hz stimulation (Klakowicz et al 2004). Additionally, M-waves during the same period remained small and statistically unchanged by the high-frequency stimulation train, suggesting the extra-contractions were not generated by changes in stimulation intensity or at the muscle level. Based on our data it is difficult to attribute extra torque generation in the ankle plantar flexors entirely to spinal pathways when the M-waves, a product of direct motor axon stimulation, appear to be driving the contractions during muscle stimulation. Similarly in the wrist flexors, the EMG traces shown in Figure 8 (C & D) indicate M-waves to be the prominent wave-form during the 20 Hz stimulation. However, previous studies using muscle stimulation clearly show extra contractions are

abolished during nerve block proximal to the stimulation site (Collins et al. 2001, 2002), thus affirming the importance of afferent input to the spinal cord even during muscle stimulation. Interpretation of the EMG during muscle stimulation does not exclude facilitation of torque output from spinal neurons; however, it suggests motor unit discharge during the stimulation may not be time-locked to the stimulus, therefore implying the presence of asynchronous motor unit recruitment sustained by their own intrinsic properties (plateau potentials). Asynchronously discharging motor units have previously been cited as a potential mechanism underlying the extra contractions (Collins et al. 2001, 2002; Nickolls et al. 2004), and have been observed during force increases during tendon vibration (Lang and Vallbo 1967; Burke and Schiller 1976). Thus, spinal reflex facilitation may not be a prerequisite for generating the extra contractions and the mechanisms underlying the extra contractions may be specific to the type of stimulation used.

The prevalence of H-reflexes and M-waves during nerve and muscle stimulation in the plantar flexors, respectively, can be explained in part by differences in stimulation location. Stimulation over the muscle was delivered close to the motor point and therefore more likely to activate a greater proportion of motor rather than sensory axons at low stimulation intensities. As a result, M-waves predominated during muscle stimulation. Conversely, stimulation over the nerve trunk, where sensory and motor axons are more evenly mixed, recruited a relatively greater proportion of sensory axons at low stimulation intensities and H-reflexes were more prevalent.

Differences in axon excitability at the stimulation site may also account for differences between nerve and muscle stimulation in the plantar flexors. The excitability

of motor axons at the motor point differs from those at the nerve trunk (Kuwabara et al. 2004). For example, more current is needed when stimulating over the motor point of abductor pollicis brevis to evoke a response equal to that while stimulating the median nerve at the wrist (Kuwabara et al. 2004; Walters et al. 2001). If more current was required during muscle stimulation to generate a test train equal to that evoked with nerve stimulation, it is likely a larger sensory volley would be delivered to the spinal cord. The larger afferent input could potentially activate asynchronously discharging motoneurons and therefore facilitate motor output (Collins et al. 2001). It is not clear if differences in axonal excitability at the motor point shown for the median nerve can be extended to the tibial nerve. Interestingly, the present data from the wrist flexors do not demonstrate the site-specific differences described above. Thus, mechanisms underlying differences in stimulation site excitability, as described previously (Kuwabara et al. 2004; Walters et al. 2001), may not have been as prominent in this experiment.

The different contributions of H-reflexes and M-waves to contractions evoked during nerve and muscle stimulation may have affected the accuracy of matching stimulation intensity. During tibial nerve stimulation H-reflexes were 3.4 times larger than M-waves. During muscle stimulation however, M-waves were 15 times larger than H-reflexes. In the individual (Fig. 2) and group (Fig. 3A) torque profiles, a sharp increase followed by a rapid decrease in torque can be clearly identified at the onset of nerve stimulation. It is most evident with low intensity tibial nerve stimulation, but is still present at the moderate intensity as well. Experiments in our lab have demonstrated a large H-reflex (34 % M_{max}) evoked by the first stimulus pulse of 20 Hz stimulation can produce a concurrent 4% MVC torque response (Klakowicz et al. 2005). The large initial

H-reflex is rapidly attenuated thereafter, as is the torque. This trend corresponds to previous work demonstrating H-reflex reduction during tetanic stimulation due to reduced neurotransmitter release at the Ia synapse, know as post activation depression (Burke and Schiller 1976; Crone and Nielsen 1989; Hultborn et al. 1996; Schindler-Ivens and Shields 2000; Taborikova and Sax 1968; Van Boxtel 1986). A large initial H-reflex is likely to account for the peak torque measured during nerve stimulation test trains and is capable of producing torque outputs commensurate to those used for matching stimulation intensities. Conversely, the H-reflex is absent with muscle stimulation and the initial deflection in torque is absent in the contraction profiles: Without the large initial H-reflex during muscle stimulation, the torque produced during the test trains will require greater current to achieve the torque evoked by nerve stimulation.

The unequal influence of H-reflexes and M-waves on a given level of torque production (i.e., the target torque values during the test trains) may have been greater for muscle stimulation, and could be responsible for the amplitude differences between nerve and muscle stimulation. Comparisons, however, between low intensity muscle stimulation and moderate intensity nerve stimulation, when the torques generated before the 100 Hz bursts were similar (see Fig. 3) demonstrates muscle stimulation was still more effective in generating the largest extra contractions.

Lower vs. Upper Limb Stimulation

Although the presence of extra contractions has been documented in lower limb, the extent to which they are present in the forearm has not previously been tested. Extra contractions were present in the wrist flexors, as in the plantar flexors. However, differences between stimulation types observed in the ankle plantar flexors were absent in

the wrist flexors. This may relate to the large influence of H-reflexes during contractions in the soleus evoked with tibial nerve stimulation. During this experiment, H-reflexes in the wrist flexors were small and their presence sporadic. Unlike in the soleus, H-reflexes in the FCR are typically generated at higher M-wave amplitude than in the soleus and are therefore reduced in amplitude as a result of antidromic collision in the efferent axon (Aymard et al. 2000). As discussed previously, the H-reflex is capable of producing large contractile responses at low stimulation intensities. During low intensity stimulation of the median nerve, H-reflex amplitudes were much smaller, 8.5 times for the three subjects analyzed, than concomitant M-waves (*also see* Fig. 8C), and therefore less likely to influence torque output during the test trains. The compromised influence of the H-reflex is demonstrated in the torque profiles (Figs. 4 & 5), as the initial deflections in torque observed with tibial nerve stimulation are absent with median nerve stimulation. Compared to the plantar flexors, intensities in the wrist flexors were more closely matched and potentially fewer differences were observed between nerve and muscle stimulation as a result.

The magnitude of extra contractions evoked in the plantar flexors during low and moderate intensity muscle stimulation was 9 and 3 times larger than contractions evoked in the wrist flexors at similar intensities. With nerve stimulation, extra contractions were slightly larger, but not statistically different during low and moderate intensity stimulation between the lower and upper limb muscles tested. Greater levels of cortically imposed presynaptic inhibition (PSI) on flexor carpi radialis Ia afferents, compared to soleus spindle (Ia) afferents has been observed in neurologically intact humans (Meunier and Pierrot-Deseilligny 1998). The 1ms pulse-widths used in this experiment were

selected to preferentially activate Ia afferents (Veale et al. 1973; Hugon 1973; Panizza 1989) and their input to the spinal cord is imperative in producing the extra contractions (Collins et al. 2001). Thus, PSI will have a very strong influence on the input received by the α-motoneuron and will greatly influence the resulting motor output. The absence of statistically different magnitudes of extra contraction between the plantar flexors and wrist flexors with nerve stimulation is not explained by varying degrees of PSI in the upper and lower limbs. It is possible that larger responses, evoked with higher nerve stimulation intensities, would more clearly demonstrate a difference between the upper and lower limb.

Mechanisms

Motoneurons, once thought of as passive integrators of synaptic input, are now known to possess intrinsic membrane properties, such as persistent inward currents (PICs) generated by low-voltage activated Ca^{2+} channels and Na^{+} channels that produce sustained depolarizations and discharge rates that outlast the initial input (Schwindt and Crill 1980; Bennett et al. 1998a; Hultborn 2002; Heckman et al. 2005). Prolonged depolarizations caused by PICs are known as plateau potentials and have been suggested as one mechanism underlying extra contractions in studies similar to this one (Collins et al. 2001, 2002). The extra contractions have been associated with plateau potentials because of their dependency on high-frequency input to the spinal cord through large diameter afferents, in addition to residual EMG outlasting the stimulation and evidence of asynchronous motor unit discharge (Collins et al. 2001). Plateau potentials induced with high-frequency input has been demonstrated in animals (Lee and Heckman 1998; Bennett et al. 1998a) and indirectly in humans (Collins et al. 2001) using electrical stimulation

and tendon vibration (Kiehn and Eken 1997, Gorassini et al. 1998, 2002). High-frequency afferent input to the spinal cord is capable of recruiting motoneurons able to fire autonomously, thus demonstrating a hallmark of plateau potentials (Gorassini et al. 2002). The present data fit with the idea of plateau potentials occurring in motoneurons as the additional recruitment of motoneurons firing independent of stimulation frequency could explain the increased torque we observed during the burst stimulations once the stimulation was reduced from 100 to 20 Hz.

Post-tetanic potentiation (PTP) could also contribute to the extra contractions observed. Sources of PTP can be found both centrally and peripherally following repetitive electrical stimulation. Centrally, PTP at the Ia synapse results in the enhancement of excitatory post-synaptic potentials (EPSPs) (Hirst et al. 1981). Peripherally, PTP of the muscle fibers enhances torque profiles (O'leary et al. 1997). A proposed mechanism for PTP at the Ia synapse is a reduced probability of failure to release neurotransmitter from the presynaptic membrane, coupled with an increased likelihood of multi-quantal release (Hirst et al. 1981). Repetitive stimulation of Ia afferents has been shown to facilitate EPSPs of motoneurons, and may be one factor in maintaining motoneuron excitability (Hirst et al. 1981). Alternatively, PTP of the muscle fiber is attributed to greater myosin light chain phosphorylation caused by increased Ca^{2+} release from the sarcoplasmic reticulum (Abbate et al. 2000; Duchateau and Hainaut 1986; Grange et al 1993).

From the data collected in this experiment, it is difficult to ascertain which mechanism was the prime contributor to the extra contractions. However, PTP of the muscle fibers seems least likely since previous studies have shown an absence of the

extra contractions during nerve block (Collins et al. 2001). If PTP of the muscle fibers was a factor in generating the extra contractions, one would expect to see the characteristic torque increases even in the absence of input from spinal neurons. It therefore seems likely mechanisms underlying the extra contractions are spinal in origin. Whether the specific mechanism is plateau potentials, PTP at the Ia synapse, or a combination of the two remains unclear.

Conclusions

The implementation of high-frequency, wide pulse-width, stimulation parameters has been suggested for use in NMES rehabilitation modalities such as functional electrical stimulation FES (Collins et al. 2001, 2002). This experiment is an initial step towards implementing such an idea. The results demonstrate that when stimulating the ankle plantar flexors, muscle stimulation produces larger peak torques; and the greatest extra contractions that are more consistent between contractions and stable within a contraction compared to nerve stimulation. Moderate intensity muscle stimulation was effective in generating the largest absolute torques; however, the largest extra contractions (relative torques) were produced with low intensity muscle stimulation. When stimulating the wrist flexors, extra contractions were evoked with each type of stimulation but peak amplitudes, extra contractions magnitudes, consistency, and stability were similar between them. Based on the EMG data nerve and muscle stimulation of the plantar flexors appears to operate through different neural pathways, despite a common ability to generate the extra contractions. In the wrist flexors, the pathways responsible for producing the extra contractions were similar. This study is the first to demonstrate the presence of extra contractions in the forearm muscles, an important finding given that

FES is often used to stimulate muscles acting about the wrist to assist in grasping tasks. Many factors are yet to be evaluated with respect to using these stimulation parameters in FES applications. Future work will need to focus on the assessment of motor unit recruitment to test the hypothesis that motor units recruited through central pathways during the extra contractions are slow twitch in nature. Additionally, the assessment of extra contraction fatigue compared to contractions evoked by conventional stimulation parameters; and the capability of extra contractions assisting in the completion of functional tasks should be explored as well. We propose that high-frequency, wide pulse-width, electrical stimulation may avoid the rapid muscle fatigue associated with current FES technologies and improve muscle atrophy treatment by recruiting muscles fibers not normally activated by electrical stimulation, and will thereby advance contemporary FES technology.

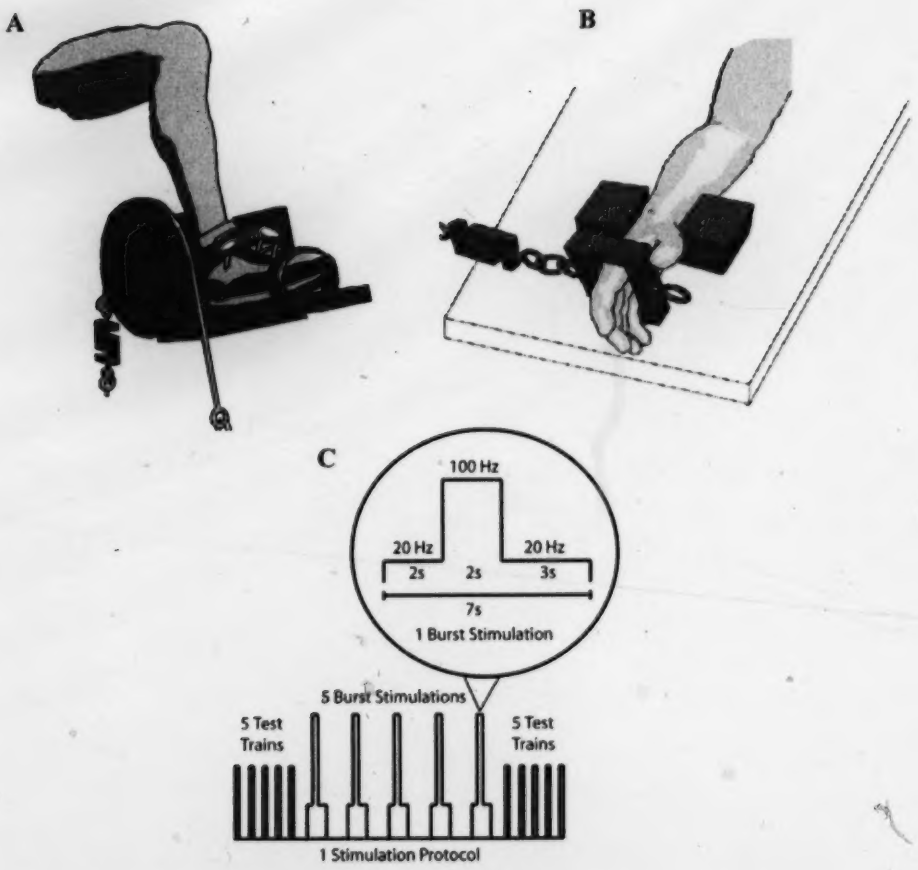

Figure 1. Schematic diagram of the experimental apparatus used for the lower limb (A) and the upper limb* (B). Torque was measured with a strain gauge and each limb was fixed to ensure isometric contractions. Schematic of stimulation protocol during a single trial (C). Individual test trains were separated by five seconds and individual burst stimulations were separated by ten seconds. A single burst pattern is depicted in the balloon above the last burst stimulation. **Adapted from Carroll et al. 2005.*

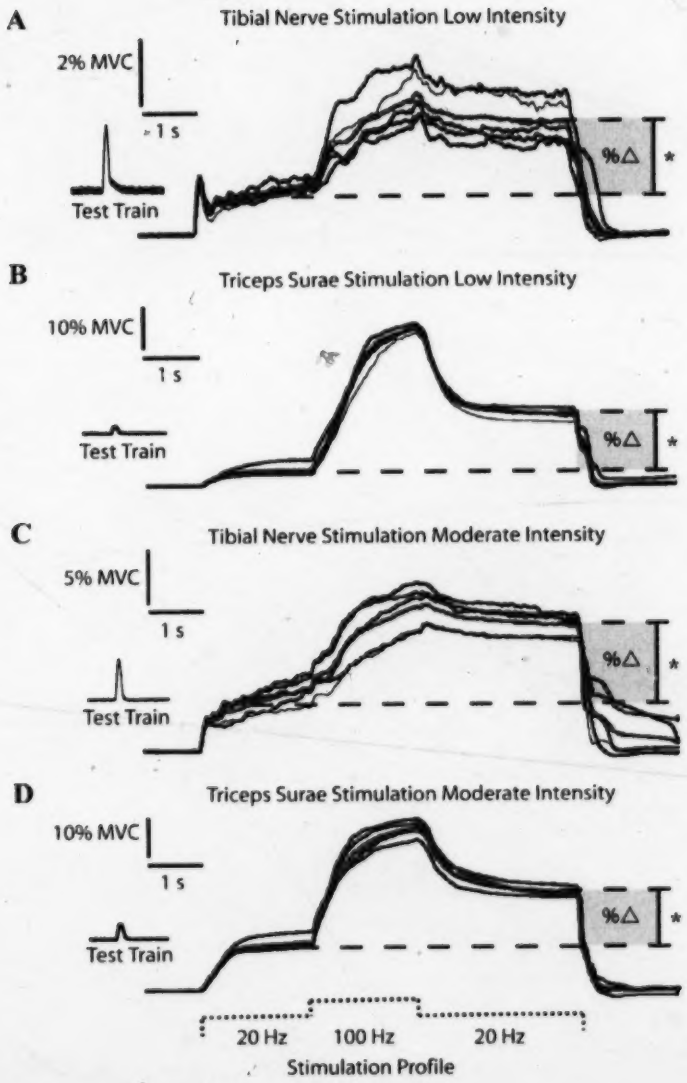

Figure 2. Torque recorded from a single subject during tibial nerve and triceps surae stimulation at low (A & B) and moderate (C & D) stimulation intensities. Grey traces denote the mean of the five torque profiles (black traces). Grey regions between dashed lines represent the amount torque increased after, compared to before, the 100 Hz stimulation period during the burst (%Δ). Significant torque increases denoted by asterisks: (A) 205 %Δ, $P = 0.002$, (B) 397 %Δ, $P < 0.001$, (C) 175 %Δ $P < 0.001$, and (D) 126 %Δ, $P < 0.001$. Average test train torques indicate stimulus intensity output prior to the burst stimulations.

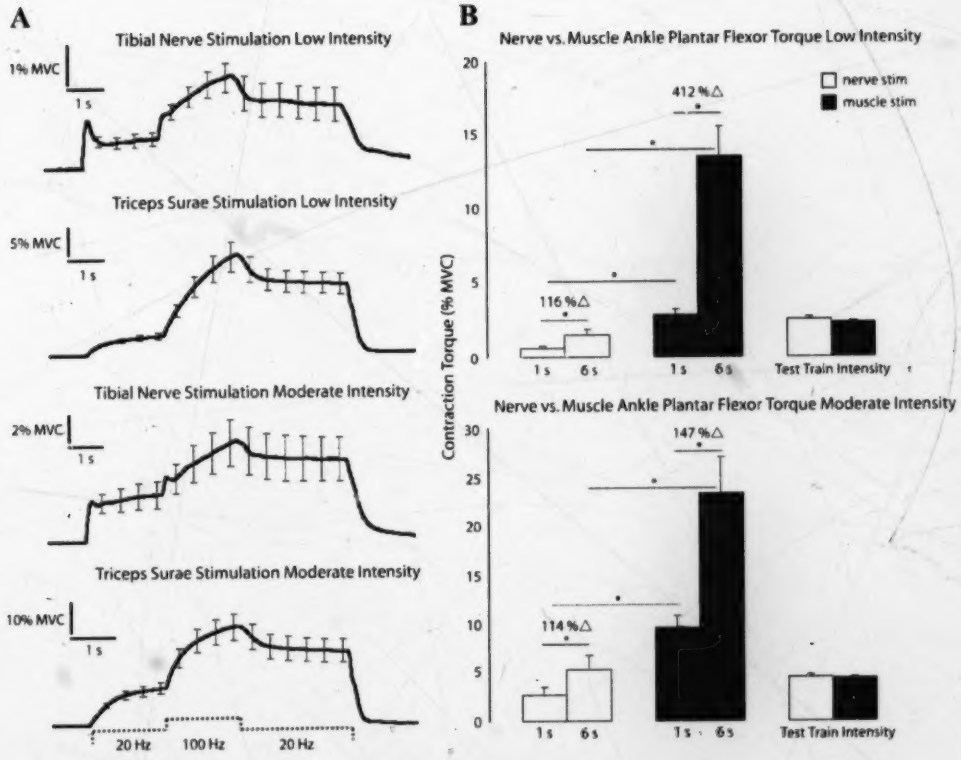

Figure 3. Group average torque (A) during 7 s burst stimulation (stimulation profile depicted by dotted line below figure) and comparisons between torques at 6 s and 1 s (B) for tibial nerve and triceps surae stimulation at low and moderate intensities. Significant torque differences are identified by asterisks. Muscle stimulation at low and moderate intensities produced significantly larger absolute torques at 1 s ($P = 0.0002$ and $P = 0.003$, respectively) and at 6 s ($P = 0.0002$ and $P = 0.002$, respectively) compared to nerve stimulation at comparable intensities. Significant extra contractions were produced with nerve stimulation at low ($P = 0.022$) and moderate ($P = 0.03$) intensities, and with muscle stimulation at low ($P = 0.0002$) and moderate ($P = 0.0007$) intensities.

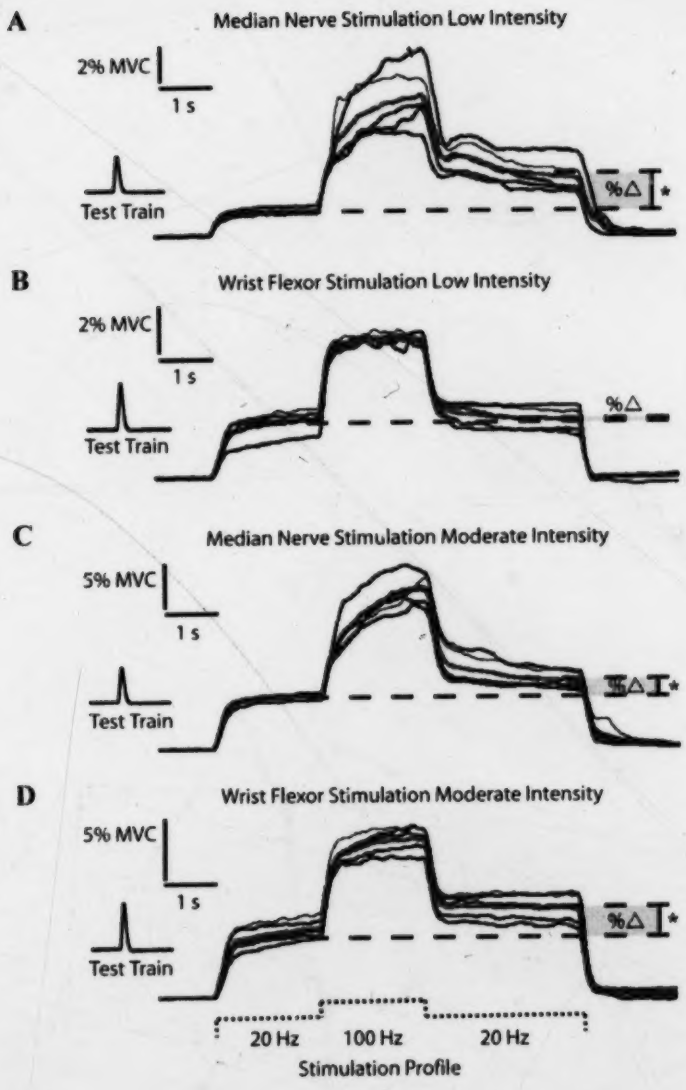

Figure 4. Torque recorded from a single subject during median nerve and wrist flexor stimulation at low (A & B) and moderate (C & D) stimulation intensities. Grey traces denote the mean of the five torque profiles (black traces). Grey regions between dashed lines represent the amount torque increased from before to after the 100 Hz stimulation period (%Δ). Significant torque increases denoted by asterisks: (A) 153 %Δ, $P = 0.003$, (B) 10 %Δ, $P = 0.59$, (C) 40 %Δ $P = 0.013$, and (D) 42 %Δ, $P = 0.016$. Average Test train torques indicate stimulus intensity output prior to the burst stimulations.

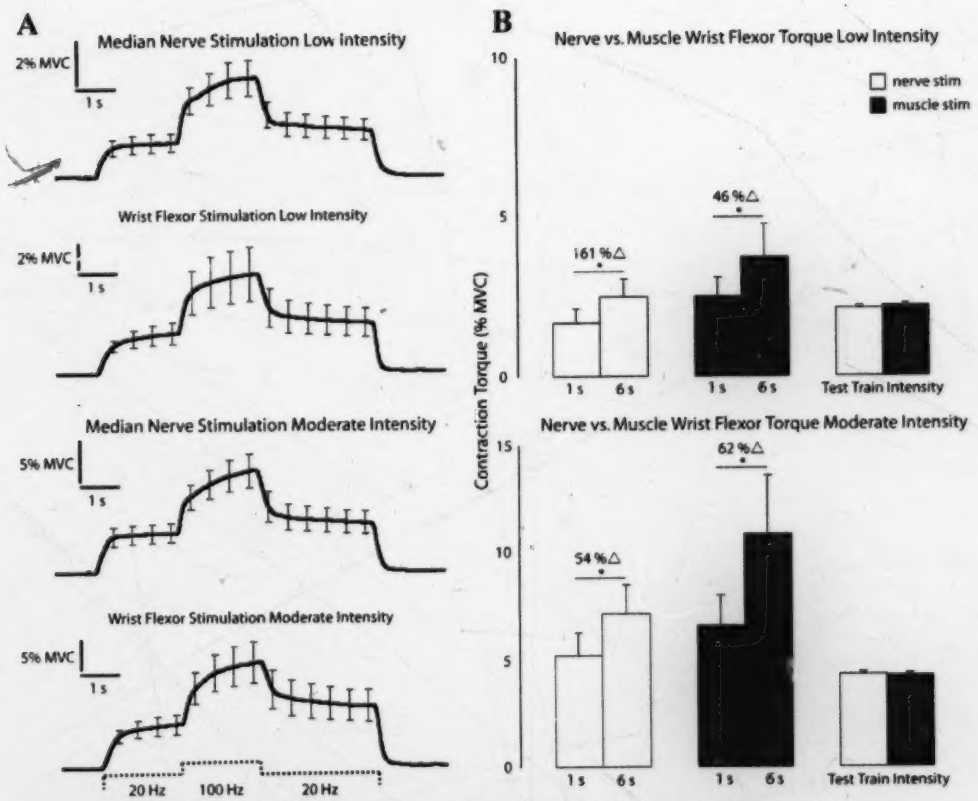

Figure 5. Group average torque traces (A) during 7 s burst stimulation pattern (stimulation profile depicted by dotted line below figure) and direct comparisons between torques at 6 s and 1 s (B) for median nerve and wrist flexor stimulation at low and moderate stimulation intensities. Significant torque differences are identified by asterisks. Absolute torque was not statistically different between nerve and muscle stimulation at either intensity. Significant relative torque increases (extra contractions) were produced with nerve stimulation at low (P = 0.007) and moderate (P = 0.005) intensities, and with muscle stimulation at low (P = 0.023) and moderate (P = 0.02).

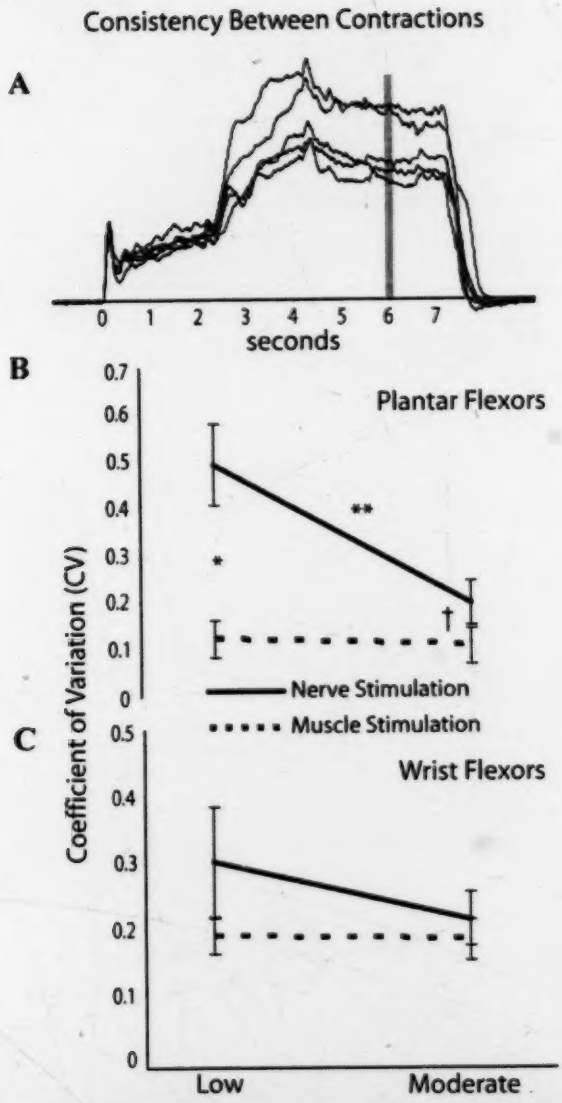

Figure 6. Contraction consistency as measured by the CV between 5 consecutive burst stimulation evoked torque profiles measured at 6 s into the stimulation (A). In the plantar flexors (B), low intensity nerve stimulation was significantly less consistent than at the moderate intensity (**, $P = 0.01$), as well as low (*, $P = 0.005$) and moderate (†, $P = 0.003$) intensity muscle stimulation. In the wrist flexors (C), contraction consistency was similar for nerve and muscle stimulation at both low and moderate intensities. Solid and dashed lines indicate nerve and muscle stimulation, respectively.

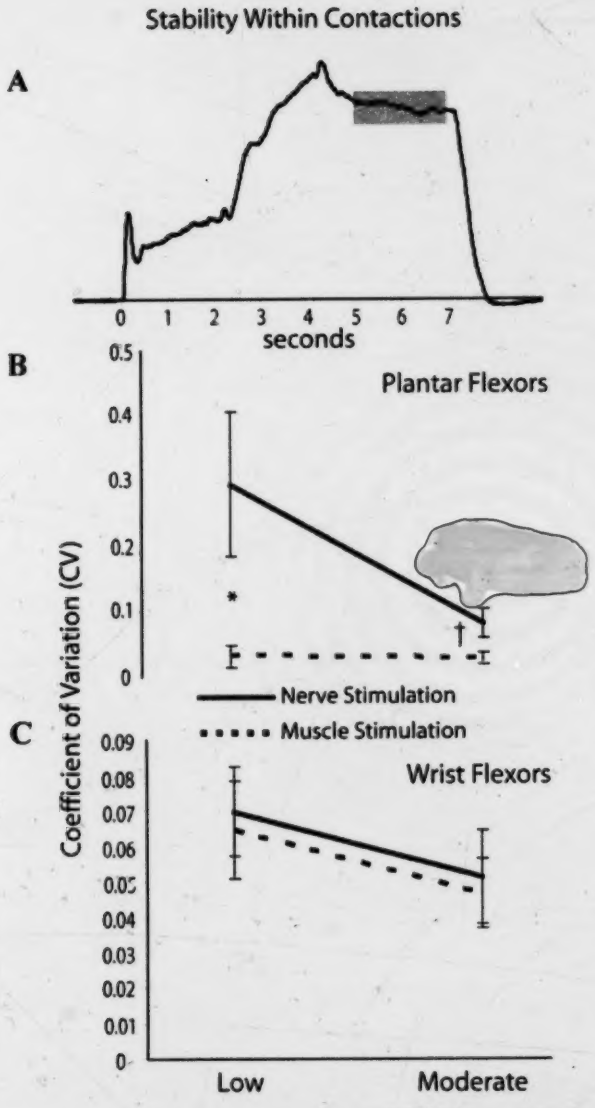

Figure 7. Contraction stability as measured by the CV over the last 2 s of stimulation (A). In the plantar flexors (B), low intensity nerve stimulation was significantly less stable than muscle stimulation at low (*, $P = 0.03$) and moderate (†, $P = 0.03$) intensities. In the wrist flexors (C), contraction stability was similar for nerve and muscle stimulation at both low and moderate intensities. Solid and dashed lines indicate nerve and muscle stimulation, respectively.

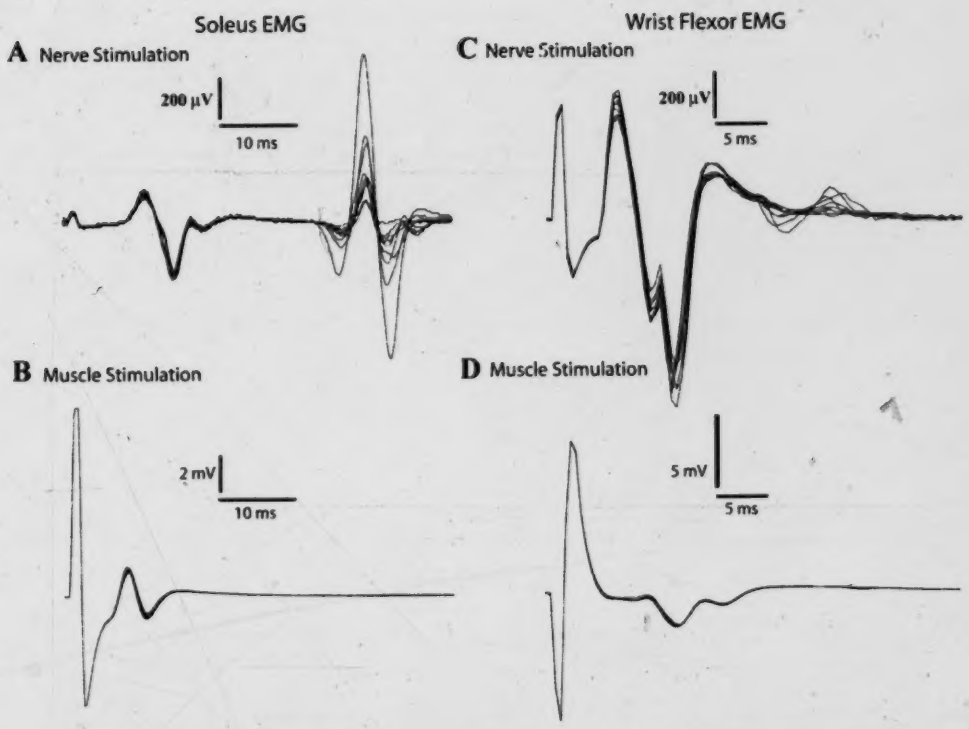

Figure 8. Raw EMG traces during low intensity tibial nerve (A), triceps surae (B), median nerve (C), and wrist flexor (D) stimulation in a single subject. Traces shown were collected during a 400 ms window spanning 200 ms before and after the 1 s mark of a burst stimulation pattern. The traces demonstrate the absence of H-reflexes, and dominance of M-waves during muscle stimulation, compared to nerve stimulation at a similar intensity. Additionally, H-reflexes were far less frequent and M-waves were more prominent during nerve stimulation of the wrist flexors, compared to plantar flexors.

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Chapter 3 - General Discussion

The purpose of this experiment was to identify the most effective method of delivering high-frequency, wide pulse-width stimulation to evoke contractions enhanced through spinal pathways (*extra-contractions*). Stimulation was applied to the tibial nerve, triceps surae, median nerve and wrist flexors at two intensities. With NMES implementation in mind, contractions were evaluated on absolute torque production, amount of extra torque produced relative to that expected from direct motor axon stimulation alone, the consistency between successive contractions, and the stability within each contraction. Moderate intensity muscle stimulation of triceps surae produced the largest torques and low intensity muscle stimulation evoked the largest extra contractions. Low intensity tibial nerve stimulation produced the least consistent and stable contractions. Extra contractions were observed in the wrist flexors with nerve and muscle stimulation at low and moderate intensities, however; absolute torques, extra contraction amplitudes, consistency, and stability were similar irrespective of stimulation type or intensity.

Previous work suggesting the benefits of extra contractions in NMES delivered stimulation over the nerve (Klakowicz et al. 2004) or over the muscle (Collins et al. 2001,2002; Nickolls 2004), without ever comparing the efficacy of each stimulation type. Furthermore, these experiments were designed to study extra contractions exclusively in either the triceps surae or tibialis anterior. The present experiment represents the first systematic comparison between stimulation over the muscle, and stimulation over the nerve trunk proximal to the nerve, to generate extra contractions in the same muscle. Additionally, this experiment is the first to demonstrate the presence of extra contractions

in the wrist flexors, and the first to quantify extra contractions in terms of their consistency and stability, which are important characteristics if extra contractions are to be used in rehabilitative or functional contexts in a variety of muscles.

Additional Mechanisms and Future Considerations

To be useful in rehabilitative applications assisting in functional tasks it is important the torque produced during extra contractions is consistent from one stimulation to the next, and stable within a given contraction. Contraction consistency and stability were evaluated by measuring the CV between five consecutive bursts for each stimulation condition, and within a single contraction over the last two seconds of stimulation, respectively. While this calculation quantified consistency and stability numerically, and has been used previously to measure fluctuations in motor output (*see* Shinohara et al. 2005), it is possible the CV for nerve and muscle stimulation in this study are negligible with respect to successful task completion irrespective of statistical differences between them. For example, tibial nerve stimulation produced the largest CV between successive contractions (least consistent). However, single subject data in Figure 2A shows the difference between five contractions is < 2% MVC which may be acceptable for successful task performance. A study using the present stimulation parameters to complete an everyday task (i.e., grasping a glass and drinking from it) would be useful in evaluating the relevance of the CV calculation as a functional measure of variability between nerve and muscle stimulation.

The underlying mechanisms of the extra contractions were mainly attributed to those of spinal origin, such as plateau potentials and PTP of the Ia synapse. Previous evidence demonstrating the absence of extra contractions during peripheral nerve block

(Collins et al. 2001, 2001), and increasing H-reflexes in the absence of concomitant changes in M-waves after 100 Hz stimulation (Klakowicz et al. 2004), speaks strongly against peripheral contributions to the extra torques. However, during muscle stimulation in the plantar flexors when the stimulus artifact was small enough to measure M-waves, an interesting trend was identified. When comparing M-waves before (@ 1 s) and after (@ 6 s) the 100 Hz stimulation train in four subjects, a significant increase in torque (213 %, $P = 0.01$) was accompanied by a significant increase in M-waves (63 %, $P = 0.03$) and H-reflexes (249 %, $P = 0.05$). Tetanic stimulation has been shown to increase the activity of the sarcolemma Na^+- K^+ pump, and thereby enhance the size of the muscle action potential (O'Leary et al. 1997; McComas et al. 1994). The resulting increase of the muscle action potential likely augments Ca^{2+} release from the sarcoplasmic reticulum, thereby enhancing actin-myosin cross-bridge forming processes, and increasing torque output (O'Leary et al. 1997; Abbate et al. 2000). The release of norepinephrine (NE) from intramuscular sympathetic nerve fibers is a candidate for increasing Na^+- K^+ pump activity, and ultimately potentiating the contractile response of the muscle (McComas et al. 1994). Peripheral nerve block would likely prevent the release of NE from these fibers, which could explain the attenuation of extra forces shown by Collins et al. (2001). Thus, the increase in M-wave amplitudes accompanied by an increase in torque suggests peripheral PTP may contribute to the extra contractions evoked by high frequency muscle stimulation. This observation is in agreement with the idea that muscle and nerve stimulation may operate through different neural mechanisms to produce the torque profiles demonstrative of extra contractions. Experiments dedicated to specifically examining changes in M-waves and H-reflexes during nerve and muscle stimulation are

to be carried out in the future, and preliminary data is to be presented at an upcoming conference (*see* Lagerquist et al. 2005).

When high-frequency pulses are added to the beginning of a sub-tetanic stimulation train, contraction forces increase in a non-linear fashion. The force increase associated with initial high-frequency pulses is referred to as the catch-like property of muscle (Ding et al. 2003). High-frequency doublets, typically separated by ~5 ms, are thought to enhance contraction forces through increasing muscle stiffness and Ca^{2+} release from the sarcoplasmic reticulum (Parmiggiani and Stein 1981; Duchateau and Hainaut 1986). Initially, the catch-like properties may seem like a potential mechanism underlying the extra contractions of the present experiment. However, it has been shown that catch-like augmentation of contraction forces is sparse in muscles potentiated by a constant frequency stimulus train delivered at 100 Hz (Binder-Macleod et al. 1998; Ding et al. 2003). Extra contractions were defined by torque that remains significantly elevated after a 2 s, 100 Hz, stimulation train. Given that catch-like inducing stimulation trains are ineffective in muscles potentiated with high stimulation frequencies, it seems unlikely they were a factor in the extra contractions observed in the present experiment.

The presence of peripheral PTP during muscle stimulation could be addressed by an experiment examining the torque output associated with maximal M-wave responses evoked with nerve stimulation, before and after a period of 100 Hz stimulation delivered over the muscle, using 1 ms pulse widths. This protocol is similar to a previous experiment were maximal M-waves were evoked before and after a 7 s period of tetanic stimulation (100 Hz, 100 μs pulse widths) over the common peroneal nerve to test for a correlation between pre-tetanic twitch-to-tetanus ratio and levels of PTP (*see* O'Leary et

al. 1997). The absence of enlarged post-tetanic responses would indicate peripheral PTP is not a factor when applying high frequency, wide pulse width, stimulation over the muscle. An increase in post-tetanic M_{max} and twitch-torque amplitudes would not only indicate the presence of peripheral PTP, it would suggest enhanced excitation of the muscle fibers at the level of the sarcolemma, and of the muscle fibers themselves at the level of the sarcomere (O'Leary et al. 1997). An increase in twitch-torque alone would suggest peripheral PTP is a product of enhanced actin-myosin mediated excitation-contraction coupling without amplified muscle fiber excitation (O'Leary et al. 1997). The final possibility would be an increase in post-tetanic M-waves without increased twitch-torques, indicating enlarged M-waves do not correspond to increases in torque after high-frequency stimulation.

Closing Remarks

This experiment set out to identify the most effective method of evoking extra contractions in the triceps surae and wrist flexors. With respect to NMES, the results suggest muscle stimulation is best suited for evoking extra contractions in the plantar flexors, while extra contractions were evoked similarly in the wrist flexors regardless of stimulation type. The next logical step is to apply the stimulation parameters used here into an NMES device and evaluate the efficacy of the extra contractions in both functional (i.e., grasping, standing) and therapeutic (i.e., muscle atrophy prevention) usage. From a basic research perspective the results yielded an interesting finding in that nerve and muscle stimulation appear to operate through different neural pathways to produce the extra contractions. The dominance of M-waves during extra contractions

evoked with muscle stimulation in the triceps surae surely bring into question previous assumptions of orderly motor unit recruitment and increased fatigue resistance. Research focused on identifying recruitment order and evaluating fatigue resistance between extra contractions evoked with nerve and muscle stimulation will further elucidate underlying mechanisms, and provide valuable insight into the practicality of their use for clinical applications such as NMES.

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